# Investigating the effect of subsoil manuring on soil arthropods in the northern midlands of Tasmania.

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## Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma and, to the best of my knowledge, contains no copy or paraphrase or material published or written by any other person, except where due reference is made in the text of this thesis.

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**20th October 2016**

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## Contents

# Abstract

A study was carried out as part of a larger trial into the soil amelioration technique called subsoil manuring (SSM). This technique involves the use of a deep ripper to directly place organic amendments into the upper layer of the subsoil of duplex soils which have significant constraints to crop growth. In this study, soil arthropods were extracted from topsoil and subsoil samples from three sites (two irrigated and one dry land) on duplex soils in the northern midlands of Tasmania. There were a total of five different SSM treatments: control, deep-ripped only, and deep-ripped with a range of organic amendments including poultry manure and poppy seed meal. Arthropods were extracted from soil samples using a Burlese-Tullgren funnel apparatus and identified to arthropod order. Two orders, acari and collembola, were dominant and used for further analysis. The acari were further separated into the suborders mesostigmata and oribatida. Data from the sites was collected and analysed separately and then the mesostigmatid population between treatments was compared across all sites. There was a significantly higher abundance of mesostigmatid mites in the plots that had an organic amendment added to the subsoil. The dryland cereal site was more responsive to the treatments compared to the irrigated cropping sites.

# LITERATURE REVIEW

A productive agricultural soil is an ecosystem of organisms. The roots of plants, fungi, bacteria, microarthropods, earthworms and many other groups of plants and animals coexist in a complex food web. These organisms and their products are important to agriculture, and their measurement can give an indication of soil health. One size class of particular interest in this assemblage of organisms is the mesofauna, consisting mostly of the orders acari (mites) and collembola. This group of organisms cannot move soil particles and are therefore reliant upon existing pore spaces in the soil for their movement, habitat and protection (Lee and Foster, 1991, Gupta, 1994). They are therefore potentially useful indicators of the structural condition of the soil, which in turn is a soil characteristic that is highly significant to the health of the crop being grown, and a widespread constraint to crop yield in Australian agricultural soils.

Agriculture is an important industry in the high rainfall zone (HRZ) of Australia, a region defined by an annual rainfall greater than 500 mm (MacEwan, 2007), and which covers parts of southern Australia and much of Tasmania. Agriculture in the HRZ on mainland Australia is dominated by cereals (Zhang et al., 2006) and in Tasmania features a mixture of cereals and vegetable cropping (Cotching et al., 2001). Tasmania's growing season is longer and cooler than the HRZ cropping areas of the mainland, with recognised potential for increased production (Zhang et al., 2006)., Extensive irrigation and high inputs are features of Tasmania's HRZ cropping region (Lisson and Cotching, 2011). Intensive vegetable cropping in Tasmania has been carried out on the stable and resilient red ferrosols of the north-west for many decades, but increased water storage, large centre-pivot irrigators, powerful farm machinery and the high value of crop products is extending that land use into the duplex, or texture contrast, soils of the midlands, used mostly for grazing until relatively recently (Cotching et al., 2001, Zhang et al., 2006). These duplex soils present many challenges for agriculture, mostly because of the structure of the subsoil.

A technique specifically designed to ameliorate duplex subsoils, termed Subsoil Manuring (SSM), has been trialed in dry-land HRZ cereal cropping areas in Victoria, and is now undergoing trials in the northern midlands of Tasmania. The physical and chemical changes to the soil, and the impact on crop yield, have been analysed as part of the Victorian project (Gill et al., 2012), but no work has been reported on the biology of the soil after SSM treatment. This review will outline the nature of duplex soils and their limitations, discuss the Victorian SSM trials and the recent Tasmanian work, give a brief background of soil biology and its interaction with soil fertility, and examine the value of using soil microarthropods as indicator organisms to study the impact of SSM on soil structure and biology.

## Duplex Soils

Duplex soils are characterised by a strong and clear texture contrast between the A and B horizons (Northcote et al., 1975), and a subsoil that has a higher bulk density and is more clay rich than the topsoil. They often have a sodic subsoil and occupy roughly 20% of Australia’s land area (Chittleborough, 1992), dominating the agricultural landscape in southern Australia (Figure 1) (Anderson, 1992, Gardner et al., 1992). Many of Tasmania’s cultivated soils are duplex soils, yet they are generally considered unproductive soils (Belford et al., 1992, Dracup et al., 1992, Turner, 1992, Zhang et al., 2006).

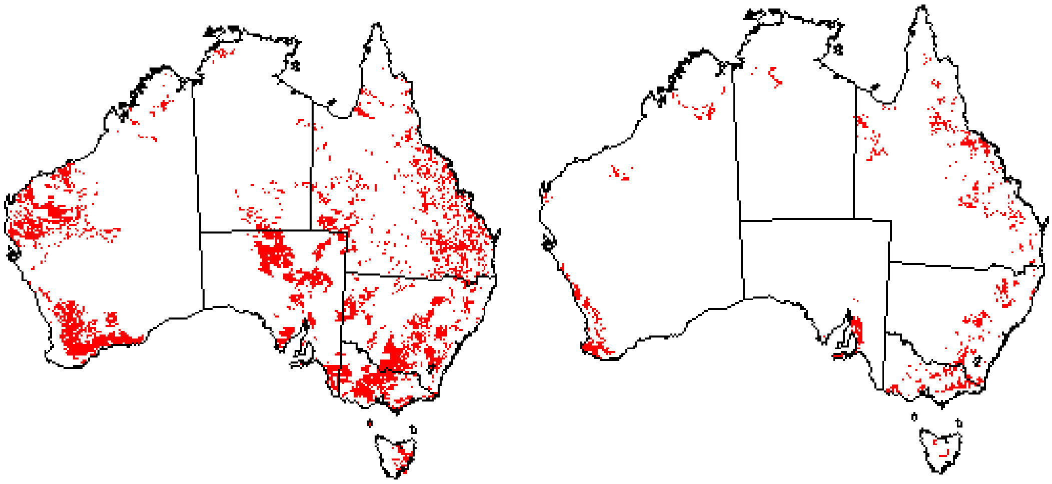


**Figure 1**. Sodosol (left) and chromosol (right) [duplex] soil distribution in Australia. (CSIRO, 2014)

### *Constraints*

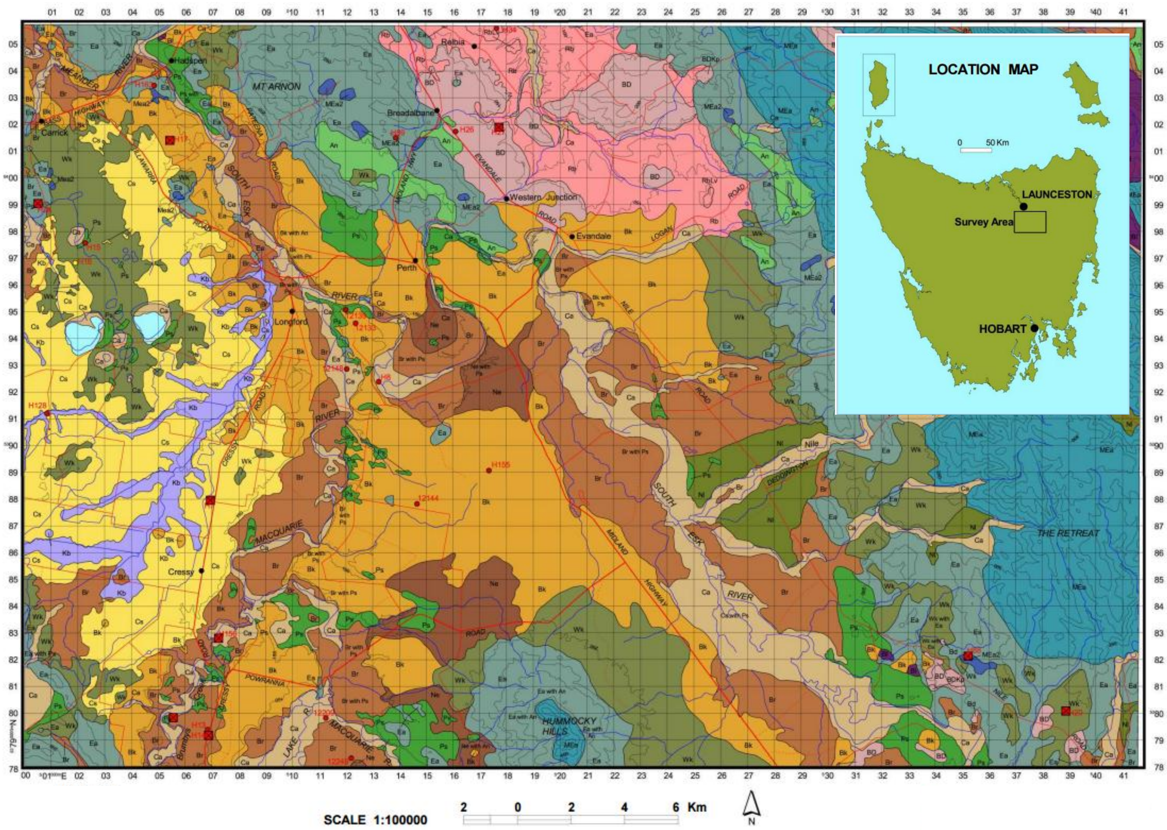
The particular challenges duplex soils present for agricultural production have been well reviewed (Anderson, 1992, Turner, 1992, Jayawardane and Chan, 1994, Zhang et al., 2006) and can be summarised as: waterlogging, mechanical impedance to root growth, compaction, sodicity and erosion.

The primary causes of these challenges are shallow topsoils and the less permeable nature of the B horizon and the high density and strength of the clay within it. This contributes to the most important constraints experienced by crops, that of waterlogging and restricted root growth (Dracup et al., 1992). Root penetration into the subsoil is prevented or limited, leading to under-developed root systems, and limiting plant access to water and nutrients, particularly subsoil water (Dracup et al., 1992, Edwards, 1992, Gardner et al., 1992).

Plants growing in conditions of compaction and mechanical impedance to root growth are more stressed and susceptible to disease (Allmaras et al., 1988, Gardner et al., 1992) and have low water use efficiency (Ellington, 1986). This may not necessarily reduce yield if there is sufficient water and nutrients in the topsoil (Wong and Asseng, 2007), but is usually detrimental.

Waterlogging is found to varying degrees in duplex soils of the HRZ (McFarlane and Cox, 1992, Armstrong et al., 2015) and is caused by low hydraulic conductivity in the dense subsoil layer – as little as 1-2 mm per day moves between topsoil and subsoil (Gardner, 1990). It is very damaging to plant roots, depriving them of oxygen, reducing or stalling water and nutrient uptake (particularly nitrogen), restricting growth, causing anaerobic respiration and ultimately, the death of parts of the root system (Allmaras et al., 1988, Gardner, 1990, Anderson, 1992). Under waterlogged conditions, soil bacteria cause denitrification, converting soil nitrate to gaseous nitrogen which is unavailable to plants, and sometimes creating toxic compounds (Allmaras et al., 1988, Gardner, 1990). The excess water can also leach plant nutrients from the soil, taking them out of the root zone of the crop (Dracup et al., 1992). Waterlogging in duplex soils can also limit vehicle and machinery use, delaying or preventing work crucial to optimal crop performance and health (Edwards, 1992).

The types of land degradation commonly associated with agriculture, such as erosion, compaction, acidification and salinization are often more acute on duplex soils than other soil types (Dracup et al., 1992). Because of shallow topsoils and poor plant growth (McFarlane and Cox, 1992), duplex soils are prone to wind erosion (Edwards, 1992), compounding the existing problems of shallow root systems and waterlogging (Dracup et al., 1992). Erosion from rain can also be significant on sloping land (Gardner et al., 1992, McFarlane and Cox, 1992). These flows of excess water can cause or exacerbate salinity, a landscape scale problem in Australian agriculture (Brouwer and Vandegraaff, 1988, Passioura, 1992). The agricultural history on many of Australia's duplex soils has left a legacy of degradation on what was already a constrained soil (Ellington, 1986, Gardner et al., 1992). Figure 2 shows how duplex soils dominate the northern midlands area of Tasmania.




| | | | |
|---|---|---|---|
| Bk | Brickendon Association | Soils developed on flat to gently undulating (0-3%) river terraces. | Chromosol |
| Ne | Newham Association | Soils on undulating and rolling (3-32%) drop-off slopes or terrace scarps. | Chromosol |
| Br | Brumby Association | Soils developed on alluvium overlying Tertiary clays on flat to gently undulating (0-3%) river terraces. | Sodosol |
| Ea | Eastfield Association | Soils developed on dolerite bedrock and colluvium on rolling to steep (10-56%) land, | Sodosol. |
| MEa | Miscellaneous Soils Related to the Eastfield | Soils developed from insitu dolerite, colluvial and solifluction dolerite debris on steep (32 - 56%) slopes of the Western Tiers and Ben Lomond Plateau. | Chromosol |
| Bl | Blessington Association | Soils developed on Trias sic sandstone on rolling and steep (10-56%) land, | Sodosol |

**Figure 2**. A soil map of an area of the northern midlands around Longford, Tasmania, with a key to the dominant duplex soils. (2014)

## *Current Approaches to Amelioration*

The effectiveness of methods of subsoil amelioration of duplex soils, detailed below, varies across seasons, sites and crop, and cannot always be relied upon to produce yield improvements (McFarlane and Cox, 1992, Jayawardane and Chan, 1994), making it difficult to justify expensive interventions. However, carefully tailored site-specific solutions that address multiple issues have been shown to overcome most of the yield limitations of these soils (Anderson, 1992).

Plant-based approaches using deep-rooted primer species and crop rotations can be important for managing duplex soils (Gardner, 1990, Gardner et al., 1992, Cresswell and Kirkegaard, 1995, Nuttall et al., 2008, McDonald et al., 2012, Real et al., 2012). Plant cultivars can also be developed with tolerances to some subsoil constraints (Graham et al., 1992).

Surface or subsurface drains are an expensive but very effective and widely applied management option for duplex soils that suffer from intermittent waterlogging (McFarlane and Cox, 1992, Brussaard, 1997, Zhang et al., 2006). They cannot, however, be used on land without some slope, limiting their application (Graham et al., 1992), and they require frequent maintenance (Zhang et al., 2006, Cotching, 2009).

Gypsum has been shown to chemically stabilise small soil aggregates (Blackwell et al., 1991) and gypsum and lime are widely used as soil amendments in various ways, such as surface applications or combining with deep ripping or slotting, to improve dense and sodic subsoils (Ellington, 1986, Jayawardane et al., 1987, Bennett et al., 2015). Trials using these methods sometimes show an increase in yield, particularly in the short term, but often have very mixed results, usually attributed to rapid leaching and careless follow-on management such as over-irrigation or heavy farm traffic over the cultivated area (Gardner, 1990, Graham et al., 1992, McFarlane and Cox, 1992, Jayawardane and Chan, 1994, Gill et al., 2008, Cotching, 2009, Gill et al., 2009, Gill et al., 2012, Bennett et al., 2015).

Soil conservation measures such as direct drilling, minimum tillage, retaining and building soil organic matter, retaining stubble, pasture leys, crop legumes, deep-rooted plant species, careful management of livestock and grazing and controlled traffic are important for developing, maintaining and restoring structure (Ellington, 1986, Anderson, 1992, Edwards, 1992, Gardner et al., 1992, McHugh et al., 2009, Armstrong et al., 2015, McPhee et al., 2015). Permanent raised beds are a notable combination of these methods, allowing good drainage and structure with no traffic on the growing area, reliably increasing yield (Jayawardane and Chan, 1994, Zhang et al., 2006). These practices improve soil water use

(Armstrong et al., 2015) and minimise the harm caused by waterlogging by allowing the soil to re-aerate more quickly (Allmaras et al., 1988, Gardner, 1990, McFarlane and Cox, 1992). Effective internal soil drainage is vitally important and relies on good soil structure with pore space continuity (Cotching, 2009).

Another approach to improving crop performance on duplex soils is the addition of organic material. Organic material and biological activity in the soil stabilises aggregates and creates channels called macropores, the crucial structural element that facilitates plant root growth, gas exchange and internal soil drainage after waterlogging (Gardner, 1990, Soane, 1990, Hinsinger et al., 2009). Increasing the size, number and stability of macropores is an important amelioration approach for duplex soils (McFarlane and Cox, 1992), and requires a combined approach of reduced compaction from traffic, irrigation and rain, chemical amelioration, reduced tillage and the addition of organic matter (Jayawardane and Chan, 1994). These measures greatly benefit the soil structure and, importantly, soil organisms such as earthworms, further improving, restoring and stabilising macroporosity (Lee and Foster, 1991, Gardner et al., 1992, McHugh et al., 2009).

Armstrong et al. (2007) found that adding composted pig bedding to a wheat crop on a duplex soil increased crop growth and had a positive effect that lasted for three years. A further study compared the application of pig bedding, raised beds and deep ripping with gypsum. All treatments had potential for long term yield increases through lowering of the water table and improving soil structure, yet like most studies on duplex soils, results were not clear cut, and environmental factors sometimes overwhelmed the treatments (Armstrong et al., 2015). Chemical fertilisers do increase crop growth and therefore organic material deposited *in situ*. However, manures and other sources of bulk organic material appear to have an advantage over chemical fertilisers as, in addition to plant nutrients they also add soil organic matter which has a beneficial effect on soil porosity, stability, biology, bulk density and plant root growth (Haynes and Naidu, 1998, Edmeades, 2003). They are also more stable and less vulnerable to leaching and throughflow (Passioura, 1992). Edmeades (2003), however, found no long-term benefits to crop production from using manures rather than chemical fertilisers.

A further development of using bulk organic material to ameliorate duplex soils is to introduce it directly into the subsoil. Graham and Ascher (1993) found that placing plant nutrients and organic material in the subsoil had a higher and longer lasting yield response, and increased root growth at depth, compared to adding them to the topsoil. A series of incubation studies (Clark et al., 2007, Clark et al., 2009) has shown that organic amendments in subsoil increase carbon, chemical fertility, aggregate formation, biological activity and root proliferation.

## Subsoil Manuring

In 2005, as part of a wheat cropping trial on a duplex soil in western Victoria, Gill et al. (2008) developed a new method of introducing organic material into the subsoil which they termed subsoil manuring (SSM). They compared the effect of deep ripping with and without the incorporation of gypsum and organic material 30-40cm below the surface using a tractor mounted ripper and delivery attachment. The grain yields from the organic amendment plots were between 27% and 96% higher than control plots. In addition, the organically amended plots showed: increased use of water from the subsoil, higher uptake of nitrogen into the plant, delayed senescence, increased macroporosity between the rip lines in the subsoil, decreased subsoil bulk density, and significantly increased root growth into deeper layers of the soil as well as between the rip lines (Gill et al., 2008, Gill et al., 2009).

The organic amendments placed into the subsoil had a number of beneficial effects. They provided a continuous supply of extra nitrogen for the crop, which increased root growth and allowed roots to colonise the subsoil, extracting water and further ameliorating the subsoil, allowing for even deeper root penetration (Gill et al., 2009). The material also provided a substrate for microbial activity, which has a beneficial effect on soil aggregation, increasing macroporosity (Clark et al., 2007). Plant roots and microbial activity are closely associated in soil and are mutually beneficial (Cresswell and Kirkegaard, 1995). Gill et al. (2009) conclude "the subsoil aggregation, and associated improvements in macroporosity, bulk density and water conductivity that occurred in the 20-40 cm soil layers, are the likely products of biological activity emanating both from the mineralization of the organic amendments, and the rhizosphere exudates of wheat roots that grew in these deeper soil layers."

The 2005 SSM treatments increased rainfall infiltration and subsoil water storage, increased available nitrogen and grew bigger plants with better root systems, allowing them to access the deeper water at the critical maturation phase of their growth (Gill et al., 2012). In 2006 and 2007, wheat and canola crops were grown in the same trial sites and, despite adverse rainfall conditions, SSM treatments showed roughly 50% increased yields compared to controls, with no further subsoil amendments applied. The ongoing results indicate that the subsoil improvements are significant and long-lasting. The authors attribute these results to increased capture and use of deep subsoil water, increased nutrient supply and reduction of subsoil waterlogging. An important result of their work was an increase in soil porosity and root growth in amended subsoils at their trial sites. This occurred not only where the amendments were placed in the rip line, but also between the rip lines, which were 50 cm apart. They speculate that the amendments greatly increased plant root growth and bacterial activity. The roots, having a continuous nutrient supply, could grow vigorously around the amendment, and also penetrate into the dense clay subsoil, creating a substrate for increased bacterial activity in the rhizosphere. Roots and bacteria secrete mucilages and polysaccharides that stabilize soil aggregates. Increased root growth also increases subsoil water extraction creating further opportunities for root penetration as well as further increased porosity and improved hydraulic conductivity and oxygen diffusion (Gill et al., 2009). As soil structure is one of the most production limiting factors of duplex soils (Gardner et al., 1992, Passioura, 1992), these biological improvements are important to explore further.

SSM has now been assessed in field trials in the northern midlands of Tasmania. Most of the trial sites were on duplex soils, although a limited number of deep sand sites were also included. Most of the Tasmanian sites are under centre-pivot irrigation, whereas the SSM work undertaken on duplex soils to date has been associated with dry land cereal production. These trials will indicate if the benefits observed in Victoria can be transferred to an irrigated annual cropping environment.

SSM trials so far undertaken have not explored the role or reaction of soil biology to the technique. Soil biology is an important and little understood element of agricultural soil health and productivity (Brussaard, 1997).

## Soil Biology

Soil is made by, and home to, an extensive ecosystem of organisms and their products. While much of the diversity, relationships and functioning of these organisms is still opaque to science, it is widely accepted that a diverse and thriving soil population leads to healthier, more resilient and more productive soil (Brussaard, 1997, Altieri, 1999, McDonald and Rodgers, 2010). Managing soils to protect the biodiversity of soil organisms may be one of the best tools we have to protect and maintain our soils and foster truly sustainable agriculture (Lee and Pankhurst, 1992).

Numerous researchers (Wood, 1989, Lee and Foster, 1991, Lee and Pankhurst, 1992, Roper and Gupta, 1995, Brussaard, 1997, Altieri, 1999, Horner-Devine et al., 2004, Kibblewhite et al., 2008) have outlined how soil organisms increase the productivity of soil by virtue of a number of functions they perform, such as:

- Decomposition – breakdown of organic material, excreting waste products and creating humus and other compounds beneficial to soil structure, fertility and water infiltration and storage.
- Nutrient cycling – soil organisms, particularly bacteria, perform mineral transformations, often making nutrients available for plant growth.
- Disease and pest suppression – a naturally diverse soil ecosystem will maintain balanced populations, helping to reduce disease and pest outbreak.
- Bioturbation – larger soil organisms and plant roots create channels and pores in the soil, allowing greater water penetration, aeration and habitat for smaller organisms. They also transport organic material from the surface into the soil profile.
- Restoration – soil organisms can repair damage and environmental degradation.

Soil organisms are closely associated with soil organic matter and so are usually more abundant in the upper layers of the soil (Burges and Raw, 1967, Gupta, 1994), although this relationship is not always observed in the field (Ives et al., 2011). The rhizosphere is the area of soil surrounding and influenced by plant roots and is an important area of heightened biological activity (Wood, 1989, Hinsinger et al., 2009). Root exudates and dead root tissue in this zone make up a significant proportion of the organic matter input into soils (Lee and Pankhurst, 1992).

In agricultural soils, more tillage generally means a lower population and diversity of soil organisms (Roper and Gupta, 1995, Altieri, 1999, Cotching et al., 2001, Paoletti et al., 2007), although Castro et al. (2015) found that while conventional tillage reduced earthworm numbers, soil microarthropods increased. Tillage increases erosion, simplifies and homogenises structure, reduces organic matter and disrupts and destroys earthworm burrows (Lee and Foster, 1991, Lee and Pankhurst, 1992, Roper and Gupta, 1995, Altieri, 1999). Practices that reduce or eliminate tillage and maintain or increase organic matter greatly increase soil biological activity (Roper and Gupta, 1995, Altieri, 1999). Reducing compaction through controlling vehicle traffic also greatly benefits soil structure (Zhang et al., 2006, McHugh et al., 2009, McPhee et al., 2015) and consequently benefits soil organisms and root penetration (Rodgers, D., unpublished data).

There are many groups of organisms in the soil, and they can be usefully divided into three groups by body length: Anderson (1988).

- *Microflora/fauna <100 µm*

  This group includes fungi, actinomycetes, bacteria, protozoans, amoebae, algae and cyanobacteria. They are only visible through a high powered microscope. Fungi has the highest biomass, but is outnumbered by bacteria, which are probably the most functionally important organisms in the soil ecosystem (Burges and Raw, 1967, Horner-Devine et al., 2004).

- *Mesofauna 100 µm – 2mm*

  This group is sometimes called microarthropods or soil arthropods and includes mesostigmatid mites, oribatid mites and collembola. They are generally visible through a stereo microscope and vary widely in size from about 0.25mm to 10mm. Many are detritivores, breaking down and consuming raw organic material, making an important contribution to microbial activity and nutrient cycling (Paoletti et al., 2007). As this group of soil organisms is the focus of this study, they will be discussed in more detail in the following section.

- *Macrofauna >2mm*

  Earthworms, ants, termites, large insects and vertebrate animals have an extensive impact on soil. Earthworms are widespread globally, and their burrows are important to the macroporosity of soil and greatly influence aeration and water infiltration (Lee and Foster, 1991, Lee and Pankhurst, 1992). Earthworm casts stimulate microbial activity in the soil and contribute to aggregation (Lee and Foster, 1991).

The abundance and diversity of soil organisms is such that sampling and studying them in any comprehensive way is effectively impossible. To observe changes in soil using soil organisms, it is necessary to choose a smaller group to act as indicator organisms (Elliot, 1997).

## Soil Arthropods

An indicator species should be widespread, representative, functionally important and responsive to physical, chemical and biological properties of the environment in a repeatable, predictable way (Elliot, 1997). Mesofauna have been shown to meet these criteria (Doran and Zeiss, 2000, Greenslade, 2007, Paoletti et al., 2007, Andres et al., 2011, Camilo Bedano et al., 2011, Cardoso et al., 2013). It is true that soil bacteria are abundant, diverse and functionally important in the soil, however, they are very difficult to study in detail, even with very technical genetic techniques (Horner-Devine et al., 2004). The mesofauna are an abundant and diverse group and occupy a key role in the food web of almost all agricultural soils in Australia, making them a valuable, though under-utilised, indicator of soil health (Crossley et al., 1992, Beaulieu and Weeks, 2007, Greenslade, 2007). They can also be identified and counted with simpler and less expensive equipment.

Mites and collembola are the most important and populous members of the mesofauna (Wood, 1989) and useful for indicating general soil biological condition (Akimov and Tarashchuk, 1998). They contribute to nutrient cycling in the soil in complex and synergistic ways (Lee and Pankhurst, 1992). Mesostigmatid mites are mostly predators of other soil organisms, although many species are unknown and unstudied (Beaulieu and Weeks, 2007). Oribatid mites and collembolans are detritivores that mostly consume fungi and microfauna (Lee and Pankhurst, 1992, Paoletti et al., 2007). Collectively, mites and collembola, referred to as microarthropods, form a convenient cohort for study due to their similar size, complex life histories and broad range of habitats (Behan-Pelletier, 2003).

This group are generally found in the surface 10 cm of the soil, and their occurrence declines rapidly with depth (Lee and Foster, 1991, Gupta, 1994). As they are unable to move soil, the microarthropods are confined to pre-existing pore spaces and do not directly (Lee and Foster, 1991, Gupta, 1994) and so are sensitive to soil quality and agricultural practices that effect structure (Beaulieu and Weeks, 2007). They do not directly influence soil structure, except through the deposition of faecal pellets which increase bacterial growth and division (Lee and Foster, 1991) and greatly increase the distribution of fungal spores (Paoletti et al., 2007) facilitating soil aggregate formation and stability (King and Hutchinson, 2007, Clark et al., 2009). The fragmentation of organic material carried out by the microarthropods is an important factor in the rate of organic matter breakdown, with a significant influence on carbon to nitrogen ratio in the soil, and therefore plant nutrition, soil carbon and other important processes (Cardoso et al., 2013, Soong et al., 2016).

A limitation of using soil arthropods as indicators is our lack of detailed taxonomy, with many species being currently unknown to science (Brussaard, 1997, Paoletti et al., 2007). It is also recognised that sampling and collection techniques for soil flora can often give an incomplete result, due to their small size and mobility (Lee and Pankhurst, 1992). However, studies have found mesofauna abundance to be strongly influenced by pore size, heterogeneity, volume and connectivity (Lee and Foster, 1991, Gupta, 1994, Akimov and Tarashchuk, 1998, Vreeken-Buijs et al., 1998, King and Hutchinson, 2007, Nielsen et al., 2008). Soil mesofauna, or microarthropods, therefore, may be used as indicators for porosity

(Greenslade, 2007, Paoletti et al., 2007). Porosity is crucial to plant growth as it facilitates aeration and gas exchange for roots and internal soil drainage and is also created and maintained by plant roots (Soane, 1990, Hinsinger et al., 2009).

Soil arthropods have great potential as sensitive and reliable comparative bioindicators (Greenslade, 2007, Paoletti et al., 2007, Camilo Bedano et al., 2011), and their study reveals important aspects of the fertility, and particularly the structural quality, of the soil (Camilo Bedano et al., 2011).

## Conclusion

Soil structure modulates soil moisture, temperature and aeration. This affects plant roots and soil organisms, which in turn create and maintain soil structure by the formation and stabilisation of soil aggregates and macropores (Lee and Pankhurst, 1992, Roper and Gupta, 1995, Altieri, 1999, Kibblewhite et al., 2008). Soil structure is a key element for plant health and productivity and is the primary limitation to root growth in the subsoil of duplex soils.

To improve the structure of the subsoil, the practices of reduced or zero-till, controlled traffic and permanent raised beds have proven benefits (Zhang et al., 2006, Peries and Gill, 2011). SSM seems to be a promising technique to add to this best-practice toolkit for duplex soils, particularly when its results are compared with the variable and often short-lived benefits achieved with various approaches that use lime and gypsum. Sale and Malcolm (2014) report that even though SSM is expensive, the significant and long-lasting effects returned positive economic results to the farms in the Victorian trials. Practices that benefit soil structure also improve soil biological activity and the abundance of soil organisms, as well as productivity and landscape scale soil security.

One of the impacts of SSM is greater root exploration of the subsoil, leading to much larger and deeper root systems (Gill et al., 2009). This expanded rhizosphere probably increases the abundance of soil organisms, and roots and organisms together work to maintain and improve subsoil structure, contributing to the long term benefits of SSM.

Soil arthropods are a useful indicator species for studying structural changes. The elements that benefit soil arthropods, such as porosity, organic material and aeration, are also important to crop growth, and so the study of soil arthropods and their response to SSM will likely yield useful insights into the effect of SSM in Tasmania, alongside the analysis of yield and soil physical and chemical properties undertaken by the broader project. These trials will further the work of producing accurate, reliable information that farmers can use to make sound decisions about the management of their business and land.

One result of their work was an increase in soil porosity and root growth in amended subsoils at their trial sites. This occurred not only where the amendments were placed in the rip line, but also between the rip lines, which were 50 cm apart. They speculate that the amendments greatly increased plant root growth and bacterial activity. The roots, having a continuous nutrient supply, could grow vigorously around the amendment, and also penetrate into the dense clay subsoil, creating a substrate for increased bacterial activity in the rhizosphere. Roots and bacteria secrete mucilages and polysaccharides that stabilize soil aggregates. Increased root growth also increases subsoil water extraction creating further opportunities for root penetration as well as further increased porosity and improved hydraulic conductivity and oxygen diffusion (Gill et al., 2009). As soil structure is one of the most production limiting factors of duplex soils (Gardner et al., 1992, Passioura, 1992), these biological improvements are important to explore further.

# RESEARCH PROJECT

## INTRODUCTION

This thesis reports on a study of soil arthropods carried out as part of a larger project investigating the effect of subsoil manuring (SSM) on Tasmanian farms in the northern midlands during 2015 and 2016. SSM is a recent agricultural innovation developed to ameliorate duplex soils for enhanced grain production in the Victorian HRZ. The Tasmanian SSM project involved six sites growing irrigated vegetables (four on duplex soils and two on deep sands) and one site growing dry land grain.

This paper addresses the research question: does the new technique of subsoil manuring have a measurable impact on soil arthropods in cultivated duplex soils in Tasmania? Three of the sites from the Tasmanian SSM project were chosen to undertake a study of the impact SSM has on soil microarthropods, or mesofauna, populations as an indicator of soil structure. Mesofauna have been shown to be bioindicators of soil structure (Greenslade, 2007, Paoletti et al., 2007), in particular, porosity (Lee and Foster, 1991, Gupta, 1994, Akimov and Tarashchuk, 1998, Vreeken-Buijs et al., 1998, King and Hutchinson, 2007, Nielsen et al., 2008), which is crucial to, and influenced by, plant growth (Soane, 1990, Hinsinger et al., 2009). Structure and porosity are major limiting factors to crop yield in the duplex soils that dominate the northern midlands and other agricultural regions of Tasmania and southern Australia (Zhang et al., 2006).

## 1. PROJECT AIMS

- To investigate differences in the relative abundance and ordinal assemblage of soil arthropod fauna in the topsoil and subsoil of untreated (control) and treated (SSM) trial plots at field sites with subsoil constraints.
- To determine if SSM has a measurable impact on soil arthropods.
- To compare the influence of the different materials used for SSM on soil arthropod abundance and diversity.

## 2. MATERIALS AND METHODS

SSM involves the placement of organic amendments into the upper layers of the subsoil using a modified deep ripper (Figure 3). The deep ripper has a large hopper mounted above two vertical tubes that are fixed to the back of the ripping tines. The amendment material flows from the hopper, through the tubes, and into the soil at the ripping depth, which is in the top 5 cm of the subsoil, or in some soils, into the A2 horizon; usually 20 - 30 cm below the surface.

**Figure 3.** The Tasmanian SSM machine in operation.

### 2.1 Site Descriptions

Three sites in the northern midlands (Figure 4) were selected from the TIA subsoil manuring trial sites, all of which have duplex soils with a loam to sandy-loam topsoil and clay-rich subsoil. Two of the sites grew irrigated vegetables, and one grew dry land grain.


**Figure 4.** The three trial sites in the northern midlands.

***"Esk Vale"***, Epping Forest

**Table 1**. Soil physical characteristics at "Esk Vale", Epping Forest.

| Depth of sample (mm) | Layer | Silt (%) | Clay (%) | Sand (%) | Org. C. (%) | Texture | Type |
|---|---|---|---|---|---|---|---|
| 0-26 | Topsoil | 18 | 17.5 | 64.5 | 2.01 | Sandy loam | Sodosol |
| 26-165 | Subsoil | 8 | 67 | 31 | 0.5 | Clay | |

This was a dry land site growing wheat. The SSM amendment was applied only eight days before the wheat crop was sown. It had some cultivation traffic over the rip lines before sowing, but after beds were formed for the crop, all traffic was controlled to the furrows between the beds. The soil arthropod sampling occurred in the following autumn, four months after the harvest of the wheat crop, as at the time of harvest, the soil was dry and hard, and likely devoid of soil organisms.

***"Bluegong"***, Poatina.

**Table 2.** Soil textural properties at "Bluegong", Poatina.

| Depth of sample (mm) | Layer | Silt (%) | Clay (%) | Coarse Sand (%) | Fine sand (%) | Org. C. (%) | Texture | ASC |
|---|---|---|---|---|---|---|---|---|
| 0-100 | Topsoil | 24 | 13 | 11 | 53 | 3.0 | Loam | Sodosol |
| 190-290 | Subsoil | 20 | 26 | 16 | 38 | 0.51 | Clay loam | |

The amendment was placed into the subsoil in late summer at "Bluegong", after harvest of grass seed. The grass was kept as pasture and grazed by cattle over the winter. The paddock was cultivated twice before the pea crop was sown late the following spring (18 Nov 2015). Sampling was carried out just prior to harvest in order to allow the subsoil amendment the entire growing season to influence the soil properties, and to coincide with the sampling of soil physical and chemical properties as part of the broader SSM project monitoring.

***"Woollen Park"***, Longford

**Table 3.** Soil textural properties at "Woollen Park", Longford.

| Depth of sample (mm) | Layer | Silt (%) | Clay (%) | Coarse Sand (%) | Fine sand (%) | Org. C. (%) | Texture | ASC |
|---|---|---|---|---|---|---|---|---|
| 0-100 | Topsoil | 20 | 16 | 38 | 26 | 4.7 | Loam | Dermosol |
| 200-300 | Subsoil | 14 | 39 | 27 | 21 | 1.3 | Clay | |

This site had raised beds, and consequently, controlled traffic management from bed formation for the rest of the season. On the 29th and 30th of January, 2016, 10 days before sampling, the site received 117 mm of rain over two days (Bureau of Meteorology) which completely waterlogged the subsoil. Sampling was carried out just before harvest of the carrot seed in order to give the amendment the full season to influence the subsoil, and to coincide with sampling of soil physical and chemical properties as part of the broader project monitoring activities.

## 2.2 Experimental design and treatments

Each site was established as a randomised complete block design, with four treatments and four replications. Treatments at each site included:

- No treatment other than the commercial practices (control)
- Deep ripped with no amendment added
- Deep ripped with poultry manure added
- Deep ripped with poultry manure and wheat chaff ("Esk Vale" only)
- Deep ripped with poppy seed meal added ("Woollen Park" and "Bluegong")

Trial plans for the three sites are shown in Figures 5 – 7.

| Replicate | Plot | SSM Treatment |
|---|---|---|
| buffer | | |
| 1 | 2 | rip only * |
| 1 | 3 | rip only |
| 1 | 4 | nil |
| 1 | 5 | nil * |
| buffer | | |
| 1 | 7 | poultry manure/wheat chaff |
| 1 | 8 | poultry manure/wheat chaff * |
| 1 | 9 | poultry manure |
| 1 | 10 | poultry manure * |
| buffer | | |
| 2 | 12 | nil |
| 2 | 13 | nil * |
| 2 | 14 | rip only |
| 2 | 15 | rip only * |
| buffer | | |
| 2 | 17 | poultry manure/wheat chaff |
| 2 | 18 | poultry manure/wheat chaff * |
| 2 | 19 | poultry manure |
| 2 | 20 | poultry manure * |
| buffer | | |
| 3 | 22 | poultry manure/wheat chaff |
| 3 | 23 | poultry manure/wheat chaff * |
| 3 | 24 | poultry manure |
| 3 | 25 | poultry manure * |
| buffer | | |
| 3 | 27 | nil |
| 3 | 28 | nil * |
| 3 | 29 | rip only |
| 3 | 30 | rip only * |
| buffer | | |
| 4 | 32 | poultry manure/wheat chaff |
| 4 | 33 | poultry manure/wheat chaff |
| 4 | 34 | poultry manure |
| 4 | 35 | poultry manure |
| buffer | | |
| 4 | 37 | rip only |
| 4 | 38 | rip only |
| 4 | 39 | nil |
| 4 | 40 | nil |
| buffer | | |

**Figure 5.** The plot layout at “Esk Vale”; plots marked with * were sampled for this study.

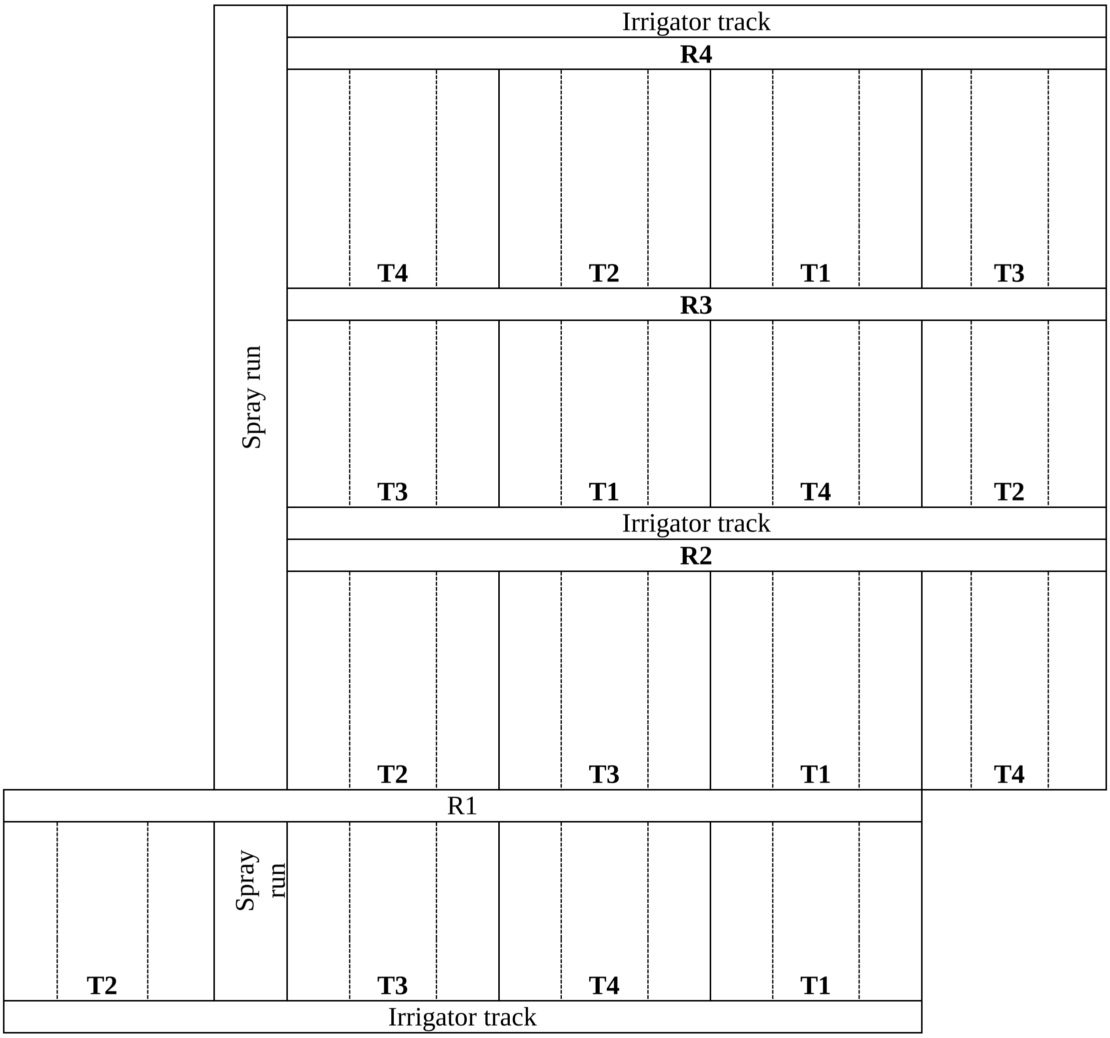



**Figure 6.** Plot layout at "Bluegong", Poatina.

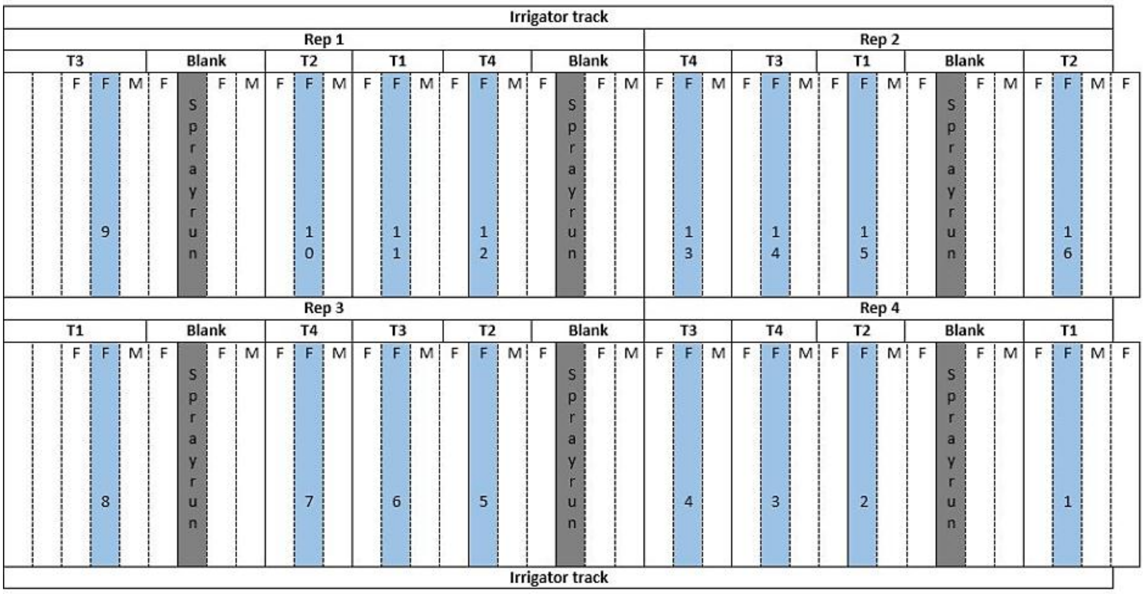



**Figure 7.** Plot layout at "Woollen Park", Longford.

**Table 4.** Site details for the three sampling locations.

| **Location** | **"Esk Vale"** Epping Forest 41°44'34" S, 147°21'05" E | **"Bluegong"** Poatina 41°46'10" S, 146°57'46" E | **"Woollen Park"** Longford 41°36'50" S, 147°03'27" E |
|---|---|---|---|
| **SSM application date** | 19th May 2015 | 11th February 2015 | 1st February 2015 |
| **SSM amendments** | Poultry manure at 40 $m^3$/ha<br>Poultry manure with added wheat chaff at 40 $m^3$/ha | Poultry manure at 35 $m^3$/ha<br>Poppy seed meal at 17 $m^3$/ha | Poultry manure at 25 $m^3$/ha<br>Poppy seed meal at 20 $m^3$/ha |
| **Arthropod sampling date** | 17th May 2016 | 20th January 2016 | 9th February 2016 |
| **Crop** | Wheat, harvested 14th January 2016. | Peas, one week from harvest at sampling date. | Carrot seed, after male plant removal, one day before windrowing at sampling. |
| **Irrigation** | Rain fed | Centre-pivot irrigated | Centre-pivot irrigated |
| **Traffic** | Raised beds, controlled traffic. | Random farm traffic | Raised beds with controlled traffic. |

**Table 5.** Key components of the chemical analysis of the SSM amendments used. P, K and Ca data from Mehlich extractions.

| **Amendments** | **C (%)** | **P (mg/kg)** | **K (mg/kg)** | **Ca (mg/kg)** | **Total N (%)** | **C:N Ratio** |
|---|---|---|---|---|---|---|
| Poppy seed meal | 51 | 5047 | 6394 | 3354 | 4.8 | 11 |
| Poultry manure | 31 | 8701 | 13129 | 12204 | 4.6 | 7 |

## 2.2 Sampling

The centre of each treatment plot was located and marked using RTK survey. The location of the rip lines was measured from this mark mid-way along the length of the plot. In each plot, a soil core was taken from the topsoil immediately above the rip line. Topsoil samples were taken upon arrival at the site, within as small a time window as possible to minimize variation in light, temperature and moisture conditions over the topsoil sampling period. A PVC core of 965.2 $cm^3$ volume was used to take the top soil samples (Figure 8).

A second soil sample was taken from the upper zone of the subsoil. This was the depth of amendment placement, and was usually 20-30cm below the surface. The nature of the texture contrast duplex soils made it clear where the subsoil began and this sampling depth was determined visually. Subsoil samples were taken with five steel cores of 205.3 $cm^3$ volume each (total sample size = 1026.6 $cm^3$) and the use of a drop hammer (Figure 8). The PVC core would have been unable to penetrate the high density subsoil, and five cores were used in order to closely match the volume of the topsoil samples.

**Figure 8.** Topsoil sampling at "Woollen Park" (left) and subsoil sampling at "Bluegong" (right).

A total of 24 samples were collected from each site, 12 from the topsoil and 12 from the subsoil. This number was determined by the number of funnels available in the arthropod extracting apparatus, and represented four treatments by three replications. All samples were emptied from the soil cores into labeled paper bags and stored in cool, shaded conditions until the extraction process was carried out later in the day.

**Figure 9.** One of the 24 funnels in the Burlese-Tullgren apparatus used to extract arthropods.

## 2.3 Sample processing

The samples were transported to the laboratory at the TIA Vegetable Research Facility at 'Forthside', weighed, and the soil placed into the Burlese-Tullgren funnel apparatus (Fig. 9). This consisted of a sieve to hold the soil, a funnel and, attached to the bottom of the funnel, a small jar of ethanol. Above the sieve there was a 50 W halogen light bulb that slowly warmed and dried the soil from the surface and caused the soil organisms to move downwards, eventually falling into the funnel and were preserved in the jar of ethanol. Under advice (Denis Rodgers, pers. comm.), the apparatus ran for 48 hours continuously. On completion of the extraction process, the temperature 1 cm below the surface of the soil sample was measured, as well as the temperature of the soil at the bottom of the sample (Table 6). The jars were then sealed and the soil weighed again, with those weights being used to calculate estimated volumetric water content. There was an exception to this process with the "Esk Vale" samples as, after 48 hours they were clearly not dry, and so ran for a further 80 hours. The cooler conditions of May, when the sampling was done, may have influenced the drying rate of the "Esk Vale" samples.

**Table 6**. The average soil temperatures at the top and bottom of the sample in the funnel apparatus at the conclusion of the arthropod extraction.

| Site | Bottom of sample (°C) | Top of sample (°C) |
|---|---|---|
| "Esk Vale" | 38.3 | 54.4 |
| "Bluegong" | 46.3 | 62.6 |
| "Woollen Park" | 42.5 | 59.7 |

**Table 7.** An estimation of the mean **s**oil volumetric water content (g/cm$^3$) of soil samples using the wet/dry weights from the extraction process.

| Site | topsoil | subsoil |
|---|---|---|
| "Esk Vale" | 29.5 | 26.3 |
| "Bluegong" | 11.0 | 8.5 |
| "Woollen Park" | 26.2 | 33.2 |

**Figure 10.** The custom-made grooved dish for counting soil arthropods.

The preserved soil organisms were transported to the laboratory at the UTAS Cradle Coast Campus. A specially designed PVC counting dish (Figure 10) provided by D. Rodgers was used with a stereo microscope at 63x magnification to count the organisms collected and identify them to order and in some cases, suborder. The identification was carried out with the aid of several resources (Brown, 1978, McDonald and Rodgers, 2010, Srivastava and Chen, 2012). Identifying this group of organisms to species level is complicated by incomplete taxonomy and outside the scope of this project and the expertise of the author.

### 2.4 Data Analysis

The program SAS v9.3 was used to carry out analyses of variances on the data. Sites were analysed separately as randomized block designs with three replicates, four SSM treatments (1, 2, 3, 4) and two depths (A, B). Proc mixed was used for all ANOVAs. Where the results were significant ($p < 0.05$) or close to significant, the pairwise comparisons using Tukey's method was used to adjust the p values. After converting all abundance figures to organisms/$cm^3$, and using only the three dominant groups of arthropods, the following factors were tested: total abundance, mesostigmatid abundance, oribatid abundance, collembolan abundance and the ratios of each group to the total abundances. The sites were then combined for an analysis of the mesostigmatid population across sites. Treatments with amendments and treatments without amendments were then combined for a comparison of mesostigmatid abundance across all sites.

## 3. RESULTS

### 3.1 Abundances

The following charts are solely of the dominant arthropod groups, the suborder oribatida (herbivorous mites), suborder mesostigmata (predatory mites) and order collembola (fungivorous/omnivorous springtails). Very few organisms of other orders were found. Numerical figures of all the arthropods found in the samples are in Appendix 1. Abundances have been converted to organisms/$cm^3$.

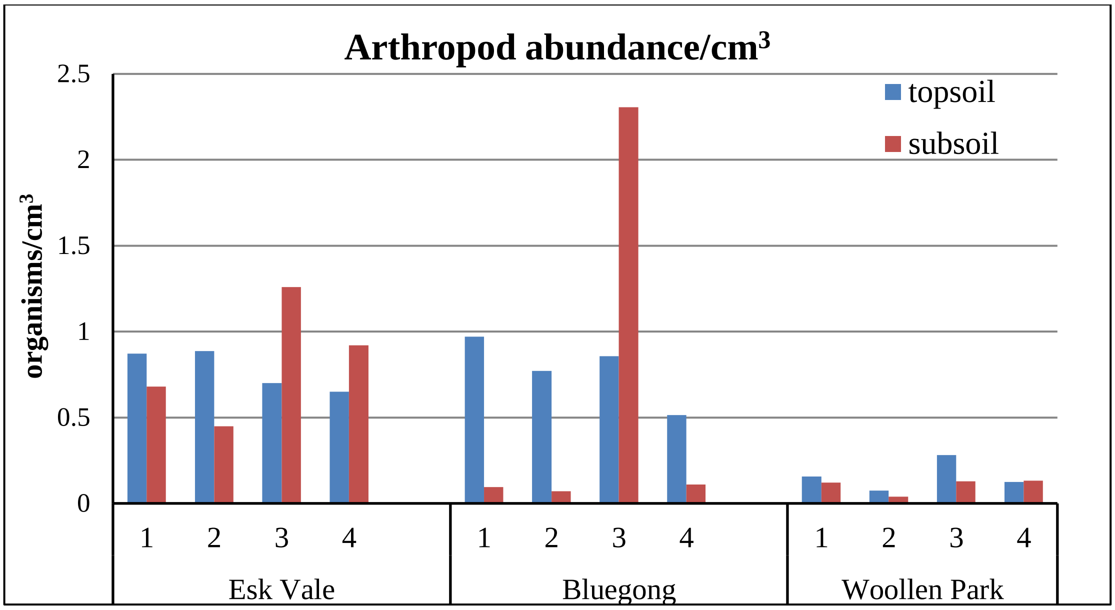



**Figure 11.** The average number of soil arthropods, extracted from each treatment at the three sites. This chart includes an outlier (Bluegong 3), and standard error was too large to mark on this chart. The treatments on the x axis are: 1. Control; 2. Ripped with no amendment; 3. Ripped with poultry manure added; 4. Ripped with poppy seed meal added ("Bluegong" and "Woollen Park") or, ripped with poultry manure and wheat chaff added ("Esk Vale").

One of the subsoil samples from treatment 3 at "Bluegong" was considered an outlier, with a total of close to 7,000 arthropods, thousands more than any other sample (Figure 11). It was removed from the data analysis and further charts.

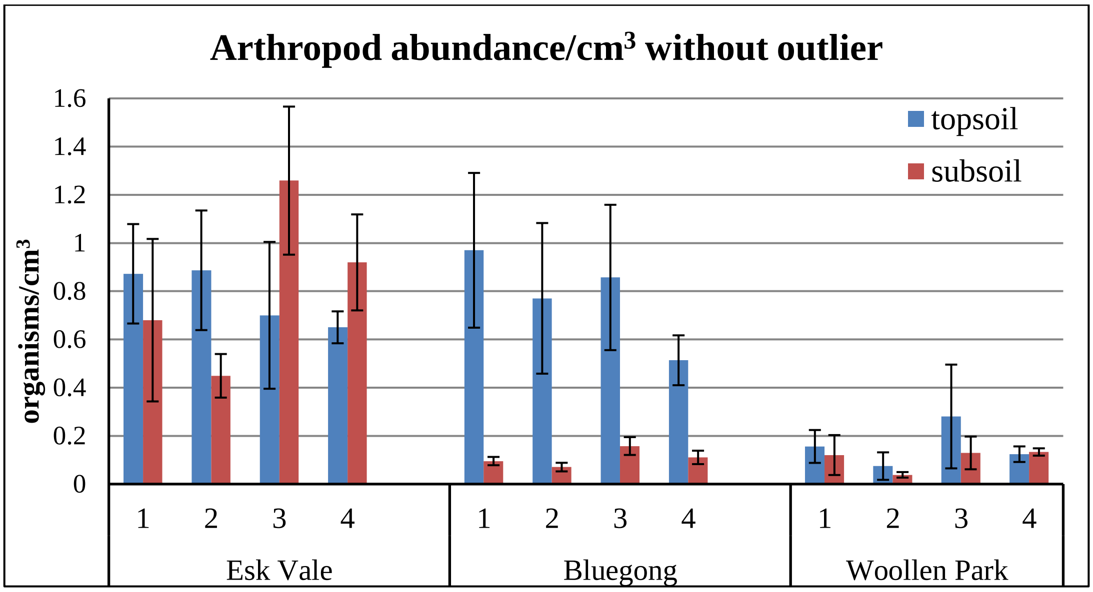



**Figure 12.** The average number of soil arthropods extracted from each treatment at three sites with standard error shown. The treatments on the x axis are: 1. Control; 2. Ripped with no amendment; 3. Ripped with poultry manure added; 4. Ripped with poppy seed meal added ("Bluegong" and "Woollen Park") or, ripped with poultry manure and wheat chaff added ("Esk Vale").

Figure 12 shows the overall abundance of the three soil arthropod orders sorted by depth of sampling and treatment. “Esk Vale” has the highest abundances in the subsoil, with subsoil arthropod abundances higher than topsoil abundances in the SSM amended treatments. All three sites have the lowest subsoil abundances in treatment 2, the rip-only treatment. It is clear that the number of organisms at “Woollen Park” was very low compared to the other sites. This chart also shows how large the standard error was in the data.

Figure 13 – Figure 15 show the abundances by order at each site. It is clear that the higher subsoil abundances at “Esk Vale” were driven by the mesostigmata and oribatida suborders, and these two groups responded to the subsoil treatments, whereas the collembolan showed a reduction in the subsoil of the rip-only treatment, but no apparent response to the SSM.

“Bluegong” had much higher numbers of arthropods in the topsoil, mostly mesostigmatids and collembolans. The mesostigmatids showed an increase in the subsoil of treatment 3, poultry manure.

“Woollen Park” showed a similar pattern to “Bluegong”, in that the mesostigmatids responded positively to the SSM treatment, but the other groups did not. The oribatida had higher numbers in the relatively undisturbed control plot.

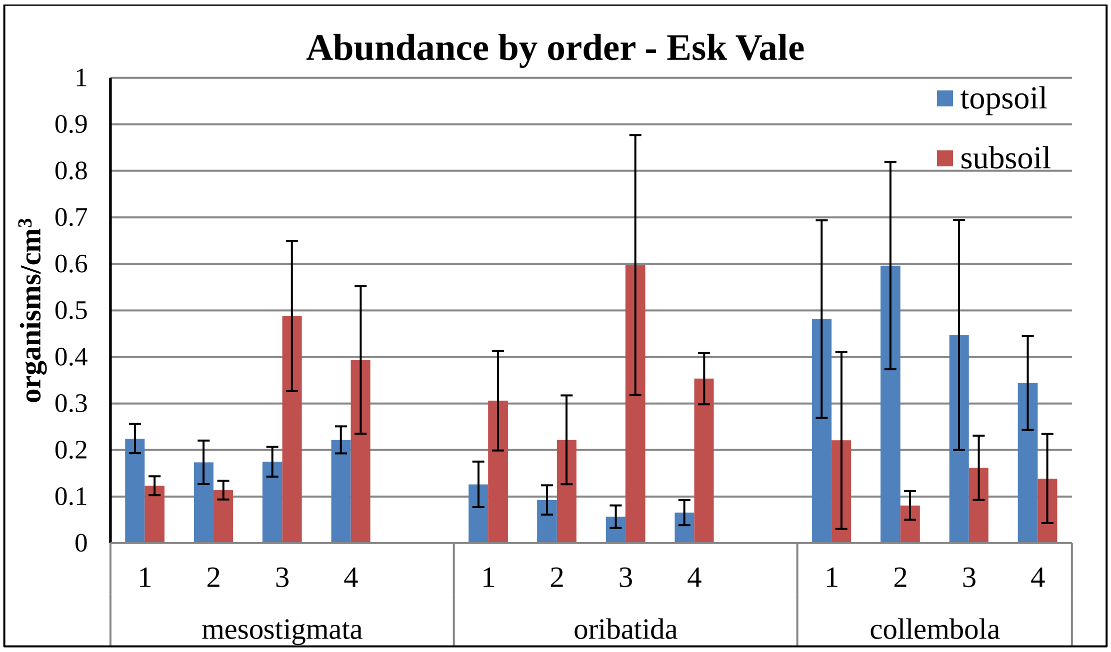



**Figure 13.** Abundances from “Esk Vale” sorted into arthropod orders showing standard error bars.

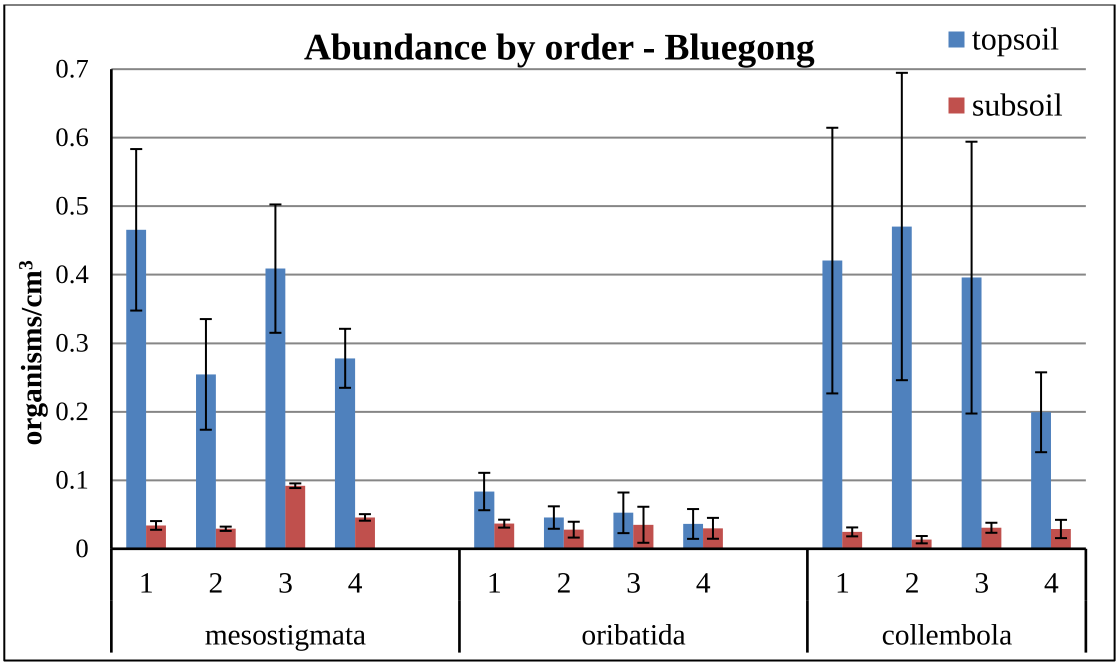



**Figure 14.** Abundances from "Bluegong" sorted into arthropod orders showing standard error bars.

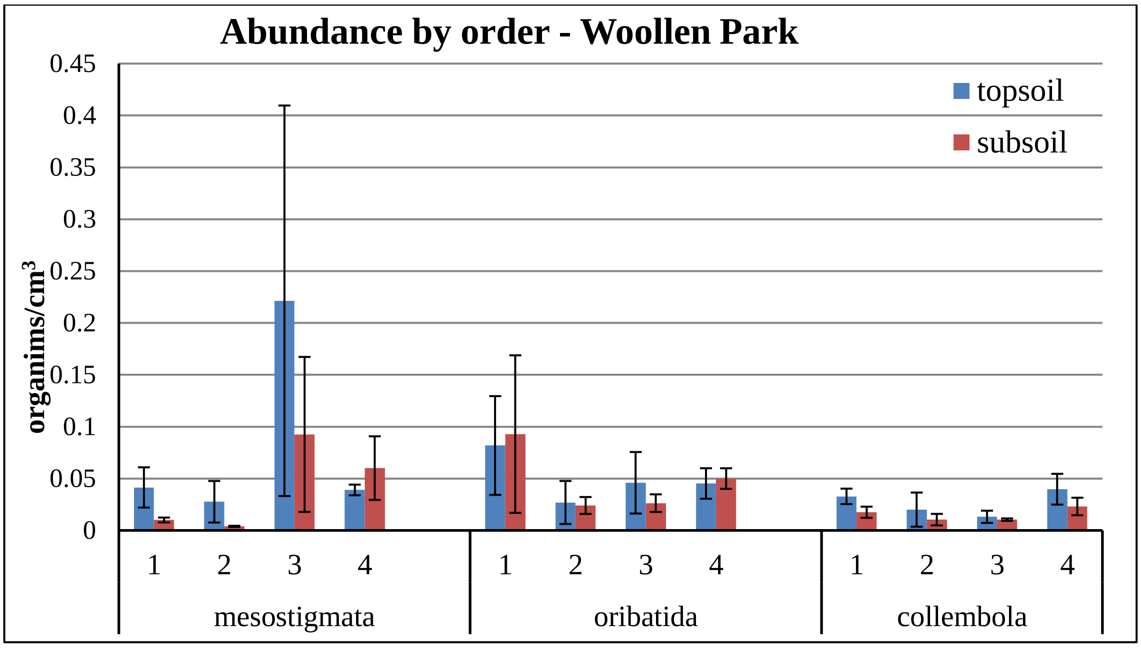



**Figure 15.** Abundances from "Woollen Park" sorted into arthropod orders showing standard error bars.

## 3.2 Ordinal composition of the subsoil

Charts (Figure 16 – Figure 18) show the proportions of each of the main arthropod orders in the subsoil samples only. All three sites showed an increased dominance of mesostigmatid mites in subsoils treated with poultry manure. “Woollen Park” had very strong oribatid dominance in the subsoil of the control treatment.

***“Esk Vale”***

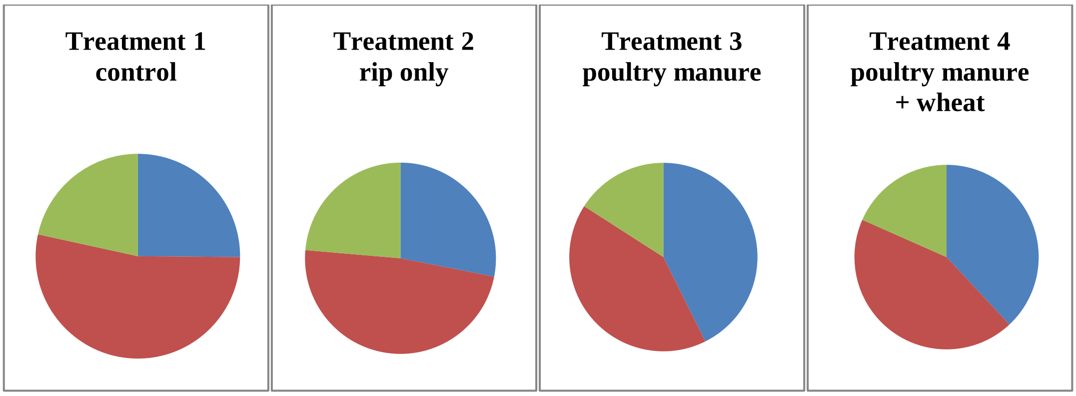



**Figure 16.** The ratios of arthropod orders in the subsoil samples at “Esk Vale”.

Mesostigmata Oribatida Collembola

***“Bluegong”***

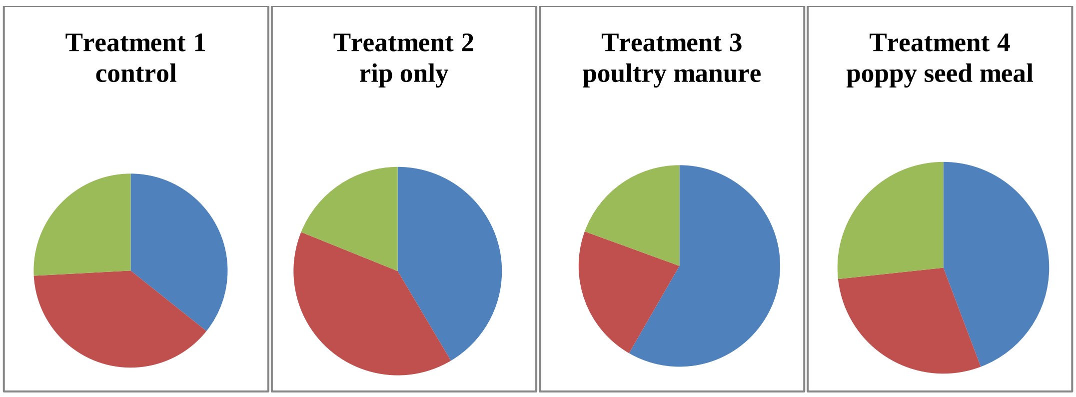



**Figure 17.** The ratios of arthropod orders in the subsoil samples at “Bluegong”.

Mesostigmata Oribatida Collembola

*“Woollen Park”*

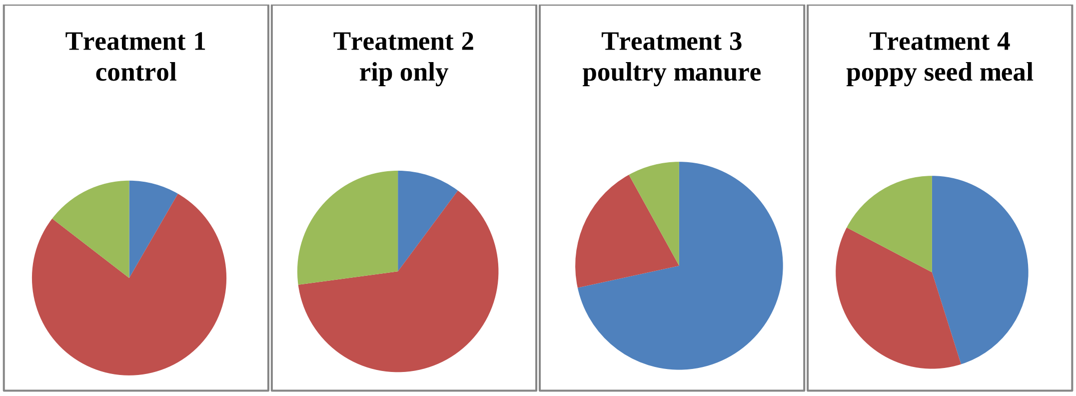



**Figure 18.** The ratios of arthropod orders in the subsoil samples at “Woollen Park”.


Figure 16 – Figure 18 show that the mesostigmatid mites became more dominant in the subsoils of the amended plots, with a stronger effect in treatment 3.

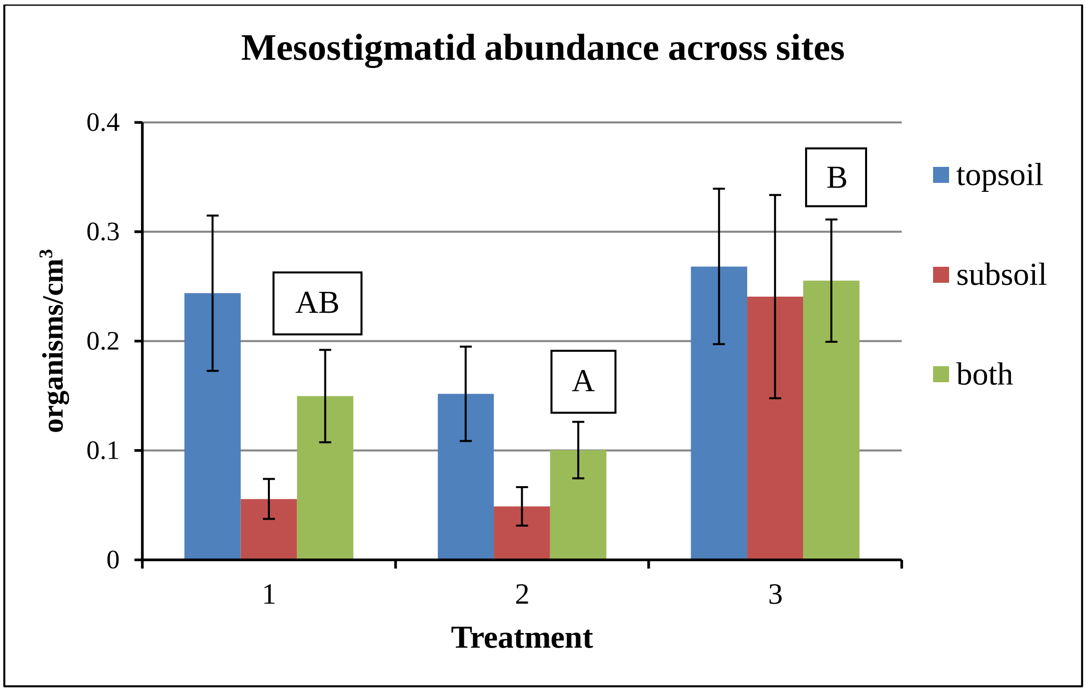



**Figure 19.** Mesostigmatid abundance across all three sites and both soil depths, including standard error bars. Different letters indicate statistical significance.

When the mesostigmatid abundances were combined across sites and soil depths, there was a statistically significant difference between treatment 2 and treatment 3 (Figure 19). Treatment 4 is not included in the chart as it would be a combination of two different amendment types. The difference in the subsoil between treatments 1 and 3 and treatments 2 and 3 was very close to statistical significance ($p$ = 0.08 and $p$ = 0.06 respectively).

Analysis of the other arthropod groups or total abundance data showed no statistically significant comparisons.

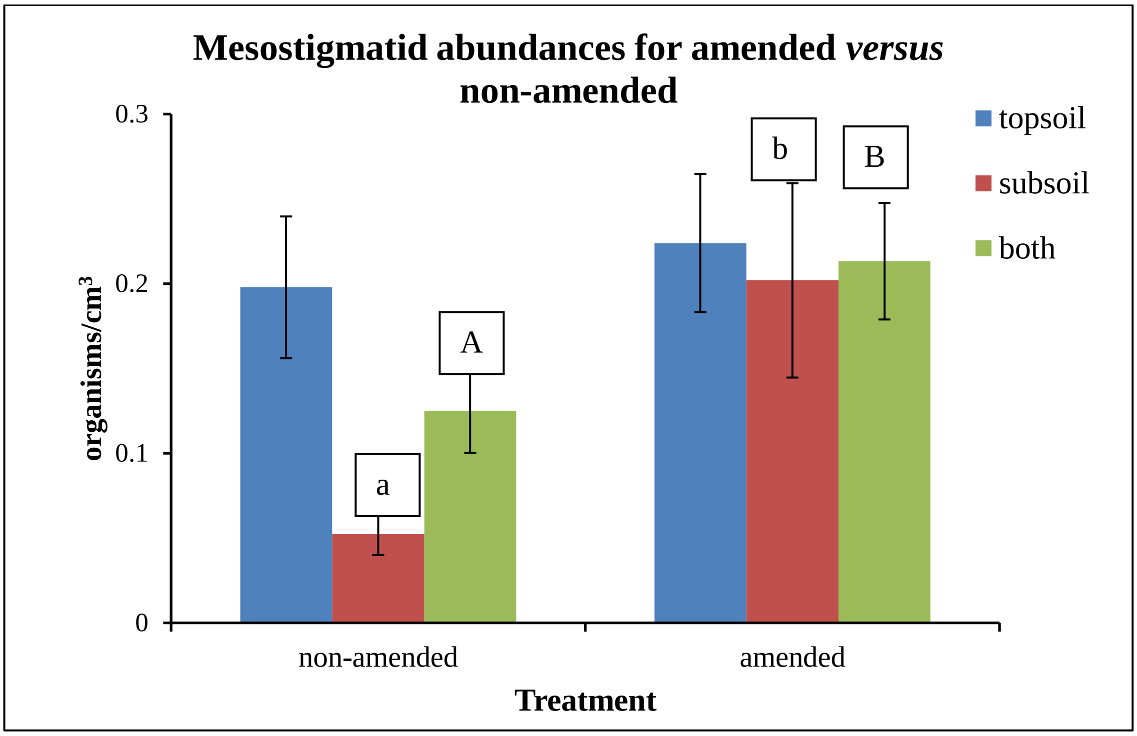



**Figure 20.** Mesostigmatid abundance in topsoil, subsoil and combined, across all three sites with treatments without amendments and treatments with amendments combined. Different letters above same depths indicate statistical significance.

A further aggregation of data pooling all amended treatments and all non-amended treatments shows that the mesostigmatid population is significantly higher across all three sites in the treatments with amendments. As above, there were no significant differences in the other groups of arthropods, nor the total arthropod abundance.

## 4. DISCUSSION

Despite the absence of statistical significance in some comparisons, the results above suggest that SSM may be altering the subsoil biology of these sites. The usual pattern of distribution for soil organisms is for higher abundances in the topsoil, where there is a higher level of soil carbon, the basic input of soil food webs (Lee and Foster, 1991, Gupta, 1994, Scharroba et al., 2016). However, in amended plots at “Esk Vale”, abundances were higher in the subsoil (Figure 12). For the oribatida at “Esk Vale”, abundance was consistently higher in the subsoil of all plots, even the control (Figure 13). The outlier from “Bluegong”, with many thousands of organisms, was also in the subsoil of an amended plot.

Other studies have shown that adding organic amendments to a site does generally increase soil arthropod numbers, an effect that varies depending on the material (Badejo et al., 1995, Bunemann et al., 2006) and manure is known to have a positive effect on microarthropods (Kautz et al., 2006). It is difficult, however, to make direct comparisons to other work, as most studies of soil arthropods analyse samples from soil no deeper than the surface 10 - 15 cm. This study was mostly interested in soil arthropods at 20 – 25 cm depth where the SSM amendment was placed. No other studies that sampled arthropods at these depths were found in the literature. Also, most studies report arthropod numbers in organisms/$m^2$, with a variable depth that is set by the soil core used and sometimes not mentioned at all (e.g. (Anderson, 1988) whereas this study has chosen to report numbers in organisms/$cm^3$.

The following discussion breaks down the results into several points in the context of the existing literature.

## 4.1 Ordinal differences

The three orders of arthropods that were the focus of this study all have a dependence on soil porosity and they usually have a close association with soil organic matter (Treonis et al., 2010, Camilo Bedano et al., 2011, Achat et al., 2012, Manhaes et al., 2013) and the rhizosphere (Achat et al., 2012). They differ in their life histories and patterns of response to disturbance and changes in the soil.

Collembolans are dietary generalists, grazing microorganisms, fungi and other organic matter (Manhaes et al., 2013). Collembola have a high rate of reproduction, short lives and relatively rapid responses to change (Behan-Pelletier, 2003). The abundances of Collembola did not react in a detectable way to any of the treatments in this study. They were more abundant in the topsoil samples, which fits the expectation that there are higher abundances of soil organisms in the topsoil (Burges and Raw, 1967, Gupta, 1994, King and Hutchinson, 2007, Manhaes et al., 2013). It is possible that high numbers of predatory mesostigmatids were reducing the abundance of collembolans, an effect observed by Schneider and Maraun (2009) who observed a 56% reduction in Collembolans with high numbers of predators present in an incubation study.

Oribatid mites are considered the most important detritivores to the soil decomposition process (Paoletti et al., 2007). They can live for as long as seven years, have a slow rate of reproduction (with egg development ranging from two months to two years in cold climates), they are generally slow to respond to changes, and are detrimentally affected by soil disturbance (Behan-Pelletier, 2003, Paoletti et al., 2007, Gulvik et al., 2008). A dominant oribatid population is associated with a stable mite population and undisturbed soil (Behan-Pelletier, 2003, Gulvik et al., 2008). Oribatid mites are very closely associated with soil organic matter, which is usually highest closer to the surface of the soil (Gulvik et al., 2008). At the Esk Vale site, numbers of Oribatid mites were higher in the subsoil samples, with the highest populations being found in the poultry manure treatment. Even the control, however, had higher numbers of Oribatids in the subsoil, a result that differs from the literature. The other two sites had fairly low numbers of oribatids, particularly Bluegong, which may be due to the low organic C content of the soil (Table 2). This group was very dominant in the relatively undisturbed subsoil of treatment 1 at “Woollen Park” (Figure 18), similar to Gulvik et al. (2008). Adult oribatid mites are thought to be immune to predation by mesostigmatid predators due to their sclerotized exoskeleton. Schneider and Maraun (2009), however, found that, similar to Collembola, their abundance decreased by 52% compared to the control when high numbers of predatory mites were present in an incubation study, probably, according to the authors, because of predation on juveniles that are less sclerotised than adults, or species that are less sclerotised.

Mesostigmatid mites have a high rate of reproduction, short lives and relatively rapid responses to change and are usually numerically dominant in agroecosystems (Behan-Pelletier, 2003). They are predatory, and feed on nematodes, collembolans, soft-bodied mites and other small insects (Behan-Pelletier, 2003, Gulvik et al., 2008). Predators are important top-down influencers on soil populations and can influence composition and diversity (Schneider and Maraun, 2009) This group responded significantly to soil treatments in this study. They showed an increased dominance in the amended subsoils (Figure 16 – Figure 18), and higher abundances in the poultry manure amended subsoils (Figure 13 – Figure 15). Mueller et al. (1990) also found that predatory mites were more dominant in buried plant material than in surface litter. Badejo et al. (1995) could not find any relationship between numbers of predators and numbers of detritivores, which they attributed to the complexity of the soil food web, whereas Vreeken-Buijs et al. (1998) and Gulvik et al. (2008) found a strong correlation to prey species. Although Vreeken-Buijs et al. (1998) found no significant relationship between mesostigmatids and land use, Gulvik et al. (2008) found a high ratio of mesostigmatid mites in more disturbed sites, as this study found, and King and Hutchinson (2007) found rapidly reproducing mites to be unaffected by cultivation.

## 4.2 Site differences

There were several major differences between the sites that undoubtedly had an impact on the arthropod populations in the soil. Soil organisms are affected by crop type, irrigation, season of sampling, cultivation and site conditions on the sampling day. For example, Vreeken-Buijs et al. (1998) found higher abundances of microarthropods in grasslands compared to forests and cropping fields, and Scharroba et al. (2016) found that the crop and litter type had a significant impact on soil organisms, even below the root zone.

The soil moisture measurements in Table 7 are not scientifically robust, as the samples were not kiln dried in the usual way, and there were minor losses of soil from the sample during the extraction process. However, they do suggest that there were differences in soil moisture conditions. “Bluegong” had much lower soil moisture than the other two sites, a possible causal factor of the low arthropod counts in the subsoil samples of that site. The moisture content at “Woollen Park” was high, as this site was inundated 10 days before sampling and was visibly waterlogged, and at “Esk Vale”, the high soil moisture was probably a feature of the season (autumn) in which sampling took place.

Sampling was carried out in summer at “Bluegong” and “Woollen Park” and in autumn at “Esk Vale”. It is recognized that soil faunal communities change through the year (Osler et al., 2000, Manhaes et al., 2013) and their numbers can fluctuate within a year more than the fluctuations between years (van Straalen, 1998). Studies have shown higher abundances in spring and autumn (Anderson, 1988) and this may be one of the reasons that “Esk Vale” had higher arthropod counts than the other sites. “Esk Vale” was a bare fallow at the time of sampling. Badejo et al. (1995) found that bare fallow had relatively low numbers of soil detritivores compared to mulched plots, only extracting, on average, 0.1 oribatid and collembolan per $cm^3$ in the topsoil. This is comparable to the topsoil oribatida and collembola numbers extracted at “Esk Vale” (Figure 13), suggesting that the abundances recorded in this trial were generally low, which is a feature of cropping soils (Vreeken-Buijs et al., 1998).

In the literature, there is a general trend of cultivation reducing abundance of the mesofauna (Anderson, 1988, Hulsmann and Wolters, 1998, Behan-Pelletier, 2003, King and Hutchinson, 2007). Figure 12 shows that there is a pattern in the data supporting this, with most treatment 2 plots, which were deep ripped but not amended, showing a lower abundance. The controlled traffic used at “Esk Vale”, compared with the more random farm traffic at “Bluegong”, may also have contributed to higher arthropod abundances. Research awaiting publication has shown a relationship between reduced compaction from machinery traffic and increased arthropod abundances (Rodgers et al. unpublished data).

Probably the most important differences in the sites were those of irrigation and crop type. The two irrigated sites – “Bluegong” and “Woollen Park” – grew peas and carrots respectively, both tap-rooted monocotyledonous plants. “Esk Vale” had grown a fibrous rooted cereal crop, wheat, under rain-fed conditions. There are several aspects of these differences in production system that affect microarthropods.

The primary influence on root depth is soil moisture (Klepper, 1991). Plants will mine the subsoil only if the topsoil layer is deficient, particularly in moisture, so that plants growing in irrigated sites with constant topsoil moisture will produce more of their roots in the surface layer (Klepper, 1991, Gan et al., 2009, Achat et al., 2012). Once the plant roots are in the subsoil, a self-sustaining ecology of microorganisms, roots and larger soil organisms could theoretically be created, such as exists in the surface layers. The numbers of arthropods found in the subsoil at “Esk Vale” may reflect this.

Dicotyledonous plants and monocotyledonous plants have different root systems and so different effects on the soil. Tap-rooted dicotyledons have been shown to create more macropores (>2mm), and contribute more carbon to the soil under irrigated conditions (Hulugalle et al., 2012).The finer and more densely rooted monocotyledons, like cereals, can create a much higher diversity of pore sizes and induce more drying of the soil with their many fibrous axes, which further creates small pores (Bodner et al., 2014). Soil arthropods show a positive relationship to the heterogeneous porosity that cereal plant roots generate (Nielsen et al., 2008), a finding which is supported by the abundance of arthropods in the subsoil of Esk Vale.

Plants can respond to mineral nutrients and organic material at depth by lateral root proliferation (Graham and Ascher, 1993, Zhang and Forde, 2000, Clark et al., 2007, Leskiw et al., 2012), also known as root plasticity, an ability that differs between species (Farley and Fitter, 1999) and is more often reported for monocotyledonous plants than tap rooted dicotyledons (Thorup-Kristensen and van den Boogaard, 1999). The roots would need to reach the SSM amendment to respond in this way, and so a moisture deficit in the topsoil is probably important for prompting deeper root exploration. “Esk Vale”, the rain-fed site, had grown a fibrous rooted wheat crop and showed the most noticeable arthropod response to SSM, suggesting that SSM may be most effective when combined with cereals and rain-fed cropping, such as in Victoria where SSM has produced large yield increases (Gill et al., 2012).

Dicotyledons, with their taproot, are generally better than monocotyledons at penetrating dense soils, although no species have been found that can reliably pierce strong duplex subsoils (Cresswell and Kirkegaard, 1995, Bodner et al., 2014). Carrot roots can reach as far as 1.6m into the soil if unimpeded, with up to 60% of those roots growing below 30 cm (Thorup-Kristensen and van den Boogaard, 1999, Westerveld et al., 2006). Gan et al. (2009) compared wheat and pea roots, and found pea roots to have much less below ground biomass, and lower root mass at depths of 60 cm and below. Fan et al. (2016) reviewed the known root distributions of common crops in temperate agriculture and showed that all had 50% of their roots in the surface 20 cm, but cereal crops had a zone of root concentration at 50 – 100 cm, and peas at 60 – 70 cm, with cereals having a deeper maximum rooting depth than peas. Carrots were not included in the review. With its ability to grow a large amount of root biomass at depth, the carrot crop at “Woollen Park” may have assisted an arthropod response to SSM if the soil had not been waterlogged. The wheat crop at “Esk Vale” was harvested months before soil sampling, but likely contributed to the high numbers of arthropods found at depth, and “Bluegong” showed low arthropod abundance in the subsoil.

## 4.3 Amendments

When comparing the two amendments used with SSM, the trend in the data of this study shows that poultry manure had more of an effect on soil arthropod abundance than poppy seed meal, which was treatment four at “Bluegong” and “Woollen Park” (Figure 12. The average number of soil arthropods extracted from each treatment at three sites with standard error shown. The treatments on the x axis are: 1. Control; 2. Ripped with no amendment; 3. Ripped with poultry manure added; 4. Ripped with poppy seed meal added (“Bluegong” and “Woollen Park”) or, ripped with poultry manure and wheat chaff added (“Esk Vale”)., or the poultry manure and wheat chaff mix which was treatment 4 at “Esk Vale”. Table 5 shows part of the chemical composition of the two amendments.

There are very few scientific studies on poppy seed meal as a soil amendment. Zaccardelli et al. (2013) found that two different seed meals, sunflower and a brassicacae species, increased bacterial activity, but only for two months. Poppy products have useful amounts of plant nutrients, particularly phosphorous and potassium, and can increase soil nitrogen in the short term (Hardie and Cotching, 2009, Ives et al., 2011). Hardie and Cotching (2009) observed poppy mulch increased soil aggregate stability, but not penetration resistance, and soil carbon only increased with very high application rates of 200 $m^3$/ha. Both Hardie and Cotching (2009) and Ives et al. (2011) noted that the time frame of their respective studies, one year, was not long enough to properly observe changes in soil physical properties and soil carbon. The sampling for this study was carried out one year after SSM application and so is possibly similarly limited.

Poultry manure is a more widely used amendment in agriculture and increases plant nutrition and microbial activity (Delgado et al., 2012, Malik et al., 2013). The results of this study show that the poultry manure amendments had a positive impact on arthropod abundance, and particularly mesostigmatid mites. This is a similar finding to Atungwu et al. (2012) who observed composted poultry manure increased microarthropod populations 46.7% – 82.4%, driven mostly by predatory mites, and also reduced nematode disease and increased yield of soybeans.

Both poultry manure and poppy seed meal showed some positive effect on soil arthropods in the subsoil of these sites. There are other practical considerations for their use in the field, such as availability, cost and transport.

## 4.4 Study Limitations

Certain elements of this study have contributed to the lack of statistical significance in the results. Some could be improved for future work and some are inherent limitations of the subject matter. Many studies of soil arthropods have found very high levels of variability in the data (Osler et al., 2000, Schneider and Maraun, 2009, Treonis et al., 2010, Camilo Bedano et al., 2011) and studies of mesofauna do not reliably generate statistically significant results (Vreeken-Buijs et al., 1998, Behan-Pelletier, 2003, Schneider and Maraun, 2009). There is a case to be made, according to (Godwin et al., 2015) for using a significance of $p<0.1$, rather than the widely used $p<0.05$, in agricultural field studies. More of the comparisons in this study would have reached statistical significance if this were the case, and as those authors point out, most land managers would be satisfied with that level of probability when making practical decisions.

Soil arthropod populations fluctuate seasonally, with soil moisture and plant residue availability, and their populations can respond to changes in environment in as little as 1-2 days (Gupta, 1994). They have a patchy, non-continuous distribution in the soil profile (Brussaard, 1997), and so taking a representative sample of the soil profile is difficult.

The resolution of this study, identifying to order only, was low due to time and expertise limitations. This is not uncommon in arthropod studies Gulvik et al. (2008) and Badejo et al. (1995). Other researchers state that the most effective taxonomic level for indicators of soil condition is species (Behan-Pelletier, 2003, Parisi et al., 2005). The taxonomy of microarthropods, however, is far from complete, particularly in the southern hemisphere (Behan-Pelletier, 2003, Paoletti et al., 2007).

The Burlese-Tullgren funnel apparatus is a widely used tool to extract soil arthropods, and the available model had 24 funnels which limited the number of samples that could be taken, thereby limiting the replication degrees of freedom in the analysis. Advice on the funnels' operation was followed, but it was later found in the literature that extraction should run for at least five days (Parisi et al., 2005). It was not known at the time that the original advice given was based on experience with red ferrosol soils, which are free-draining, and should have been amended to adapt to the duplex soils in this study. The "Esk Vale" samples were run for a much longer time than the other two sites, due to obvious moisture remaining in the samples after 48 hours, and the higher abundances from that site could be partly due to the extra extraction time. The samples from the other sites did appear dry to the author at the conclusion of the extraction process, so it is only speculation that a longer extraction time may have resulted in improved extraction.

"Woollen Park" was a promising site for response to SSM due to the use of permanent raised beds and controlled traffic – a management approach found to decrease bulk density and improve soil structure (McPhee et al., 2015) and increase soil arthropod abundance (Rodgers, D. unpublished data). The major rainfall event 10 days before sampling, and the subsequent saturated conditions in both topsoil and subsoil, likely had a strong negative effect on results at this site. The subsoil waterlogging was visually obvious and widespread and the number of arthropods extracted was very low (Figure 12), reflecting the need for air-filled pores as habitat for microarthropods (Gupta, 1994).

## 5. CONCLUSIONS

Porosity is the element of structure most relevant to both soil organisms and plant growth (Soane, 1990). The results from this study support other findings that subsoil manuring (SSM) can improve porosity, and therefore conditions for crop growth on duplex soils. Subsoil improvement is easier to achieve in dryland cereal cropping than in irrigated vegetable cropping, probably because of the fibrous root system and adjacent rhizosphere of the cereal crop seeking moisture at depth in the soil profile.

Given the naturally high variability of populations, effective and reliable use of mesofauna as an indicator of soil structure on duplex soils may require a more intensive study with more samples and replications, seasonal calibration and repetitions over different seasons and with different crops. Although there is consensus that invertebrates are important for maintaining soils and their fertility, there is little agreement on which group or aspect of their population structure provides the best measurement of their functioning (King and Hutchinson, 2007, Paoletti et al., 2007). There is also a lack of observations of soil arthropods in the soil profile below 15 – 20 cm. As this study found arthropods in high numbers, even in the control treatments, at 20 – 25 cm, there needs to be baseline work carried out in order to make valid comparisons. This study found that the mesostigmatid mites showed the most significant response to SSM and so may be a good bioindicator for studies of subsoils in the northern midlands. As predators, they respond to changes in soil structure, as well as prey availability, integrating the effects of SSM.

Poor soil structure is a major impediment to agriculture in the northern midlands, but is also a widespread limitation in other regions and situations such as traffic induced compaction, industrial reclamation and land rehabilitation (Leskiw et al., 2012). Alleviating poor structure benefits the crop, the decomposer food web, which has a suppressive effect on disease and pest organisms (Treonis et al., 2010), carbon and nitrogen cycling, and plant nutrition. SSM can improve subsoil porosity and biological activity, particularly with a high nutrient amendment like poultry manure, as shown by the increase in the mesostigmatid population in this study, and so is a valuable technique for growers with subsoil limitations. The functioning of the soil ecology is the crucial element to ameliorating subsoil limitations long-term (Young and Crawford, 2004, Clark et al., 2009). Soil biology builds and maintains porosity that facilitates the ecology of the mesofauna, as well as facilitating other services of the agroecosystem and preventing landscape scale damage such as erosion, nutrient leaching and salinity.

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## Appendix 1

Total abundance counts of all organisms identified in soil samples, averaged over three replications.

| **BLUEGONG** Treatment | Control | | Ripped Only | | Ripped + Chicken Manure | | Ripped + Poppy Seed Meal | |
|---|---|---|---|---|---|---|---|---|
| Sampling Depth | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil |
| Mites and Collembola | | | | | | | | |
| Mesostigmatid (predator mites) | 449.3 | 35 | 245.7 | 30.0 | 394.7 | 2001.7 | 268.3 | 47.0 |
| Oribatid (herbivorous mites) | 80.7 | 37.7 | 44 | 28.7 | 50.7 | 67.7 | 35.0 | 30.7 |
| Collembola (springtails) | 406.0 | 25.3 | 454 | 13.7 | 382 | 298.0 | 192.3 | 29.7 |
| Coleoptera (beetle larvae) | 7 | 0.3 | 4 | - | 4.3 | 0.7 | 1.3 | - |
| Lumbricidae (earthworms) | - | 0.7 | 1.3 | 0.3 | 3.3 | 3.7 | - | 1 |
| Psocoptera (booklice) | 6 | 0.7 | 1.7 | - | 5 | 1.3 | 2.3 | - |
| Chilopoda (centipedes) | 0.3 | - | - | - | 0.7 | - | - | - |
| Nematoda | 18.7 | 0.7 | 12.7 | 0.7 | 19.3 | 0.7 | 32.7 | 1.3 |
| Symphyla | 0.3 | 0.3 | 0.3 | 1 | - | 0.7 | 0.3 | - |

| **WOOLLEN PARK** Treatment | Control | | Ripped Only | | Ripped + Chicken Manure | | Ripped + Poppy Seed Meal | |
|---|---|---|---|---|---|---|---|---|
| Sampling Depth | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil |
| Mites and Collembola | | | | | | | | |
| Mesostigmatid (predator mites) | 40.0 | 10.3 | 26.7 | 4 | 213.7 | 95 | 37.7 | 61.7 |
| Oribatid (herbivorous mites) | 79.0 | 95.3 | 26 | 24.7 | 44.3 | 27 | 43.7 | 51.3 |
| Collembola (springtails) | 31.7 | 18.0 | 19.3 | 10.7 | 12.7 | 10.7 | 38.3 | 23.7 |
| Coleoptera (beetle larvae) | 2.7 | - | 0.7 | - | 0.3 | - | 0.3 | - |
| Lumbricidae (earthworms) | 1 | - | 2.3 | 0.3 | 4.7 | 0.7 | 0.3 | 0.3 |
| Araneae (spiders) | - | - | - | - | 0.3 | - | - | - |
| Diplopoda (millipedes) | - | - | - | - | 0.3 | - | - | - |
| Psocoptera (booklice) | 1.3 | - | 0.3 | - | 2.7 | - | 4 | 0.7 |
| Chilopoda (centipedes) | 1.7 | - | 1 | - | 2.3 | - | 1 | - |
| Nematoda | 1.3 | 0.7 | - | 0.3 | - | - | 1.7 | 0.3 |
| Symphyla | - | - | - | - | - | 0.3 | - | - |
| Hemiptera | 1.7 | - | 0.7 | - | 1.7 | 0.3 | 0.7 | 0.7 |

| **ESK VALE**<br><br>Treatment | Control | | Ripped Only | | Ripped + Chicken Manure | | Ripped + Chicken Manure/Straw | |
|---|---|---|---|---|---|---|---|---|
| Sampling Depth | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil | Top-soil | Sub-soil |
| Mites and Collembola | | | | | | | | |
| Mesostigmatid (predator mites) | 216.7 | 126.3 | 167.3 | 116.7 | 168.7 | 501 | 214 | 404 |
| Trombidiformes (‘sucking’ mites) | 39.0 | 31.3 | 23.7 | 33.7 | 20.7 | 11.7 | 18.7 | 35.0 |
| Oribatid (herbivorous mites) | 121.7 | 314.0 | 89.3 | 227.7 | 54.7 | 613.7 | 63.0 | 362.7 |
| Collembola (springtails) | 464.7 | 226.3 | 575.7 | 83.0 | 431.7 | 166 | 332.0 | 142.3 |
| Coleoptera (beetle larvae) | 0.7 | - | 1 | - | - | 0.3 | 2 | - |
| Lumbricidae (earthworms) | - | - | - | 1 | - | - | - | - |
| Enchytraed (small worms) | - | 1.7 | - | 0.3 | - | 1 | - | 1 |
| Araneae (spiders) | 1.3 | - | 3 | - | 4 | 2 | 3.7 | - |
| Psocoptera (booklice) | 0.3 | 1 | 1.3 | 1 | 0.7 | 0.3 | - | - |
| Nematoda | 0.3 | - | 0.7 | 1 | 0.3 | - | 0.3 | 0.3 |
| Symphyla | 0.3 | 14 | - | 2.7 | - | 3.3 | - | 1.7 |
| Hemiptera | 0.3 | - | - | 0.7 | - | - | - | 0.3 |